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Endothelial Seeding for Abdominal Aortic Aneurysms
Lessons Learned From the Past and Present

Manuel Mayr, MD, PhD; Michael T. Watkins, MD

In this edition of *Circulation*, Franck and colleagues[1] report a novel approach to modulate the growth of aortic aneurysms in an animal model by using endothelial cells (ECs) or outgrowth ECs grown in culture that were then delivered directly into an aortic xenograft before implantation. The authors report that this therapeutic approach resulted in modulation of the inflammatory process in the aortic wall and decreased the rate of growth of the xenograft aneurysms. They propose that endothelial seeding may be a promising technique for managing the growth of aneurysms in humans. This article brings back memories of the herculean efforts initiated by a number of laboratories to seed ECs on vascular grafts with the hope that this technique would improve the patency of the grafts and decrease perianastomotic neointimal hyperplasia and loss of ischemic limbs.[2,3] In vitro assessment of the growth potential of adult human sources of endothelium suggested that there was adequate in vitro growth of cells to theoretically cover the surface of commonly used grafts.[4] In vitro studies also demonstrated that human EC proliferation and morphology were modulated by both extracellular matrix and growth factors.[5] Human trials of EC seeding gave variable results, none convincing[6,7] enough to try to bring the technique to widespread use. Because of concerns that the surface of prosthetic grafts would never provide a hospitable environment for EC growth and development, the focus of these efforts changed to first lining the matrix with proteins such as fibronectin[8] or with growth factors that would potentiate EC growth[9] along the luminal surface of grafts. Eventually, as research into endothelial and smooth muscle cell biology progressed, this led to attempts at ex vivo construction of complete living cellular vessels.[10] With the previous efforts of endothelial seeding of grafts in mind, it is useful to consider the similarities and differences of the efforts of Franck et al to seed EC onto aortic xenografts. First, we should discuss the similarities.

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During efforts to seed prosthetic grafts and aortic xenografts, great care was taken to characterize the endothelial identity of the cells used for seeding. Specific immunohistochemical markers of EC identity were used in early studies of EC seeding, and in these studies of aortic xenograft seeding, flow cytometry and mRNA analysis were also used to confirm the cellular identity of cells being seeded. Although 5 million rat ECs were injected, only a few were detected in the intima 7 days after cell therapy. Quantification of the initial and subsequent loss of ECs during the first few days after seeding would have been essential. In studies of cell therapy, it is customary to perform titration experiments to characterize the temporal aspects of engraftment. From the description of the experiments, it is possible that the number of cells engrafted at days 1 and 7 were similar. If that was so, the interpretation of these results might be different.

In ex vivo experiments to seed prosthetic vascular grafts, fibroblast growth factor-1 was previously found to promote EC adherence to grafts.[9] In the aortic xenograft seeding experiments in this article, the authors attribute the benefits from cell therapy to paracrine effects and were able to confirm that basic fibroblast growth factor (bFGF) produced by rat ECs could potentiate growth of human ECs in vitro; however, in seeded grafts, mRNA for bFGF was present only transiently in the wall up to 7 days after seeding. Although it is well known that ECs can deposit bFGF into the extracellular matrix where it can influence metastasis and angiogenesis,[11] the cellular origin of the local bFGF production in seeded xenografts needs to be confirmed. The early bFGF expression colocalized with green fluorescent protein (GFP) signal in the neointima of GFP^{+/+} rat EC-treated aortas, but given the poor engraftment, is it likely that a few seeded ECs account for a >3-fold increase in vascular bFGF expression?

The relationship these authors draw between the ability of soluble rat-derived bFGF in culture medium to promote human EC growth in vivo and the ability of bFGF to modulate rat EC growth on the lumen of experimental xenografts is unfounded on the basis of the data presented in the article. There were no experiments done in vivo to block the effect of bFGF or to detect bFGF protein in the matrix of the seeded aortic xenografts. These results would be more compelling if the authors had shown that bFGF protein is deposited in the matrix of the seeded xenografts. Then, it may be plausible that transient seeding of the aortic xenografts with ECs could modulate the matrix characteristics of the xenografts to facilitate and sustain the ingrowth of native endothelium.

To prove their point about the utility of cell seeding, Franck et al also used late-outgrowth ECs to seed aortic xenografts. They harvested peripheral blood monocytes, cultured them, and then selected the colonies that had an endothelial morphology for subsequent passage and seeding experiments. The endothelial lining of blood vessels shows remarkable heterogeneity in

structure and function.[12] Surprisingly, rat ECs isolated from the thoracic descending aorta or obtained as late-outgrowth ECs from monocytes were equally effective for aneurysm repair. It is well known that macrophages can develop an endothelial phenotype and secrete growth factors such as bFGF and vascular endothelial growth factor,[13,14] which may explain their near-equivalent capability to modulate the growth of the experimental aneurysms compared with rat ECs. A more important question is, Why did the late-outgrowth ECs not outperform their mature EC counterparts? One of the promises of stem cell therapy is that stem cells offer a superior source of cells for tissue engineering because of their regenerative potential. However, just like ECs, there was no evidence for long-term residence of the seeded late-outgrowth ECs. This is reminiscent of previous studies with early-outgrowth endothelial progenitor cells. The criteria for what defines an endothelial progenitor cell in terms of surface markers remain highly controversial.[15,16] Unlike late-outgrowth ECs, endothelial progenitor cells do not give rise to ECs. Instead, the definition of endothelial progenitor cell has been obscured by the uptake of platelet microparticles by monocytes/macrophages,[17,18] and the mechanisms for the in vivo improvements observed in preclinical models after endothelial progenitor cell administration remain elusive, with paracrine effects, rather than differentiation, being the most likely explanation. Similarly, the authors in the present study speculate that injected cells secrete paracrine factors that have the potential to modify the endogenous healing process. This conclusion is not specific enough from a mechanistic standpoint.

A common practice in cell therapy experiments is to compare the cell treatment with a saline injection or a plain medium control. Cell culture media are supplemented with growth factors, including bFGF, and, in the case of ECs, additional supplements such as heparin. Although cells were cultured in serum-free media before injection to ensure that they contained no extraneous proteins, cells can selectively retain proteins from the bovine serum supplement.[19] ECs, in particular, show a substantial carryover of bovine proteins in the conditioned medium.[20] Their glycocalyx, which is located on the apical surface of ECs, will bind growth factors such as FGF. Thus, the seeded cells could act as a delivery vehicle for growth factor supplements from the in vitro culture. To further demonstrate that the tissue repair is indeed dependent on the secretory activity of the seeded cells and not a result of a cross-contamination of the cell preparations with growth factors from the serum supplement, conditioned medium from the EC culture and non-EC cells should be used as controls.[19] Although the authors demonstrate that rat ECs contain bFGF proteins in vitro, the protein data were not underpinned by mRNA expression levels comparing bFGF expression in rat ECs and late-outgrowth ECs. Published experiments show that healing of experimental aneurysms in rats may be modulated by systemic[21] and local administration of bFGF.[22,23] On the basis of the literature and the findings in this article, it would be of great interest to know whether coating bFGF onto aortic xenografts provided the same protection against aortic dilation as the process of cell seeding.

An important difference in the concept of endothelial seeding of prosthetic grafts in animals versus humans that is relevant to these rodent experiments is the fact that given enough time, most dog, sheep, and baboon models of arterial graft healing would develop an endothelial lining with all the appropriate anti-inflammatory and thromboresistant properties as the native vessels. However, in human vascular grafts, a complete healing process has never been documented.[24,25] Substantial ongoing adherence of platelets to human vascular grafts has been detected years after graft implantation,[26] suggesting that these grafts never develop an antithrombotic layer even though they develop a well-defined pseudointima.[27] Detailed study of the differences between human and animal EC growth in vitro revealed the need to grow human endothelium on a collagen substrate that allowed adherence of growth factors, heparin, and matrix proteins such as fibronectin.[5,28] Canine, bovine, and ovine ECs have no absolute requirement for specific matrix proteins or growth factors to achieve serial growth in vitro.

The authors are to be commended for seeking a potential solution to halt the growth of aneurysms in humans. This is a significant clinical problem, and the potential complications associated with interventions for aneurysms are not trivial despite major advances with minimally invasive endovascular techniques. However, for the experiments by Franck et al to have significant clinical relevance, it is important to show that the extracellular matrix lining their aneurysmal xenograft replicates the environment found in human abdominal aortic aneurysms. Proteomic methodology has previously been used for the first detailed analysis of the extracellular matrix in human abdominal aortic aneurysm and identified markers of pathological extracellular matrix remodeling related to matrix metalloproteinase-12 activity.[29] This methodology could be used to assess the proteome of the aneurysmal xenograft for comparison with the proteome of human aneurysms. If the matrix of the rat xenograft aneurysm is capable of binding growth factors that the human aneurysm matrix cannot, these studies will remain relevant to rats rather than humans.

Disclosures

None.

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